J. ENVIRON. SCI. HEALTH, B31(6), 1171-1187 (1996)

SOLID-PHASE EXTRACTION AND GC ANALYSES OF SELECT AGRICULTURAL PESTICIDES AND METABOLITES IN STREAM WATER

Key words: Solid-phase extraction, Pesticides, Metabolites, GC, Stream water

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ABSTRACT

The occurrence of agricultural pesticides in surface waters around the USA has created a concern over the status of safe drinking water. Solid-phase extraction (SPE) or liquid-liquid extraction (LLE) is usually employed to concentrate trace levels of pesticides in water samples to concentrations that are measurable with advanced chromatographic instruments. We describe here a SPE and capillary gas chromatographic (GC) procedure to extract and concentrate trace levels of select agricultural pesticides and metabolites from stream water. Our SPE and GC method provides high sensitivity, with recoveries between 85% to 95%, and high reproducibility for 9 of the pesticides studied. The described method provided marginal recoveries of 19 and 60% for the atrazine metabolites.

INTRODUCTION

The long-growing season associated with the mild climate of the southeastern Coastal Plain region of the USA allows annual intensive agricultural production. Typically, fields are double- or triple-cropped with agronomic and horticultural crops that require a diverse mixture of pesticides as part of an annual crop management plan. The potential exists for a portion of these pesticides to migrate to shallow ground and surface waters because of the high rainfall, high water table, sandy soil textures, and low soil organic matter contents in this region (Kellog, 1993).

The need to protect drinking water supplies from pesticides and other organic contaminants has prompted the continuous development of analytical methods for pesticide extraction and quantification. Extensive monitoring for pesticides in stream water in a USDA demonstration watershed in the Coastal Plain region of southeastern North Carolina has been in progress for over three years. Since this region has heavy annual applications of pesticides and the potential exists for pesticide migration to surface waters, a method was needed that would allow for the extraction and determination of multiple pesticides in a single water sample.

Pesticide measurements in water are often below typical instrument detection limits, and water is not a compatible matrix with GC analyses. Therefore, determination of pesticides in a water matrix traditionally requires a concentrating step using LLE or SPE prior to chromatographic analyses. Standard extraction methods for pesticides in a water matrix (USEPA, 1988) involve LLE, which uses hazardous organic solvents. The use of LLE to concentrate pesticides has the disadvantage of being time consuming.

The use of SPE for extraction of pesticides from water has recently become the method of choice. It is faster and does not require large amounts of organic solvents, which subsequently reduces the cost of solvent disposal. The C_{18} SPE cartridge has been typically used for extraction of triazines (Nash, 1990; Thurman et al., 1990), chloroacetamides (Macomber et al., 1992; Aga et al., 1994), and phenoxy herbicides (Wang and Huang, 1989) from a water matrix. In most of these studies, pesticide recovery percentages from spiked samples typically ranged from 80% to 100%.

We developed a rapid laboratory procedure using a SPE cartridge and a capillary GC equipped with a nitrogen-phosphorus detector (NPD) to extract and quantify select agricultural pesticides and their metabolites. The pesticides chosen for this study (Table 1) are typically used for pest control in North Carolina (Gianessi and Puffer, 1990). All the chemicals (except metalaxyl) are herbicides labeled for usage on agronomic crops as recommended by North Carolina State Extension (1994). Metalaxyl is a fungicide labeled for use on tobacco. In addition, two metabolites (deethylatrazine and deisopropylatrazine) from the breakdown of atrazine were included because of the high frequency of detection in ground and surface waters of the USA (Thurman et al., 1992).

MATERIALS AND METHODS

Chemicals

All pesticides and metabolites (Table 1) used in the study were obtained from Chem Service Inc. (West Chester, PA[1]) and had purity labels > 99%. HPLC grade

[1] Mention of a trade-mark, proprietary product, or vendor is for information only and does not constitute a guarantee of warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

TABLE 1
Select Agricultural Pesticides Investigated in Study.

Pesticide	Abbreviation	Chemical Name
alachlor	ALA	2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide
ametryn	AMET	N-ethyl-N'-(1-methylethyl)-6-(methythio)-1,3,5-triazine-2,4-diamine
atrazine	ATR	6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine
cyanazine	CYAN	2-[[4-chloro-6-(ethylamino)-1,3,5-triazin-2-y1]amino]-2-methyl-propanenitrile
deethylatrazine	DEA	6-chloro-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine
deisopropylatrazine	DIA	6-chloro-N-ethyl-1,3,5-triazine-2,4-diamine
metalaxyl	METAL	N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester
metolachlor	METOL	2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide
metribuzin	METRIB	4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one
prometon	PMT	6-methoxy-N,N'-bis(1-methylethyl)-1,3,5-triazine-2,4-diamine
prometryn	PRYN	N,N'-bis(1-methyethyl)-6-(methylthio)-1,3,5-triazine-2,4-diamine
terbuthylazine	TBA	6-chloro-N-(1,1-dimethylethyl)-N'-ethyl-1,3,5-triazine-2,4-diamine

methanol (MeOH), acetonitrile (AcN), and ethyl acetate (EtOAc) were obtained from various commercial sources. All H_2O used in this study was filtered using a Millipore Milli-Q filtration system (Millipore Corp., Marlborough, MA).

Preparation of Standards

A working stock solution (10 μg mL^{-1}) of each pesticide dissolved in AcN was prepared. Aliquots from these stock solutions were used to prepare GC standards of 0, 25, 75, 150 and 300 μg L^{-1}, which were brought to volume with EtOAc. A 100-μg L^{-1} pesticide spiking solution (contains all compounds except TBA) was prepared by pipetting aliquots from the working stock solutions and diluting to volume with MeOH. A 5100-μg L^{-1} TBA spiking solution (as an internal standard) dissolved in MeOH was also prepared. All solutions were refrigerated (3 °C) while not in use. Although a MeOH matrix could have been used, AcN was used as the matrix solution for the working stock solutions since some of these solutions were later used in other pesticide experiments. The varying ratio of AcN:EtOAc in the GC standards did not affect GC operating conditions or the peak area ratios.

GC Apparatus and Chromatographic Conditions

All analyses were carried out on a Varian 3600 CX GC (Walnut Creek, CA) fitted with a Varian NPD, a split/splitless capillary injector, and a Varian 8200 autosampler. The GC column was a Restek (Bellefonte, PA) Rtx-35 (crossbond 35% diphenyl, 65% dimethly polysiloxane), 30-m x 0.25-mm I.D., and 0.25-μm film thickness. Detector gas flow rates were hydrogen at 4.5 mL min^{-1} and air at 175 mL min^{-1}. The helium make-up gas was supplied to the detector at 25 mL min^{-1}. Helium carrier gas was supplied to the column at a flow rate of 1.8 mL min^{-1}. The injector and detector temperatures were maintained at 260 and 300°C, respectively.

It was necessary to develop two GC methods (referred to as method I and II) to resolve the compounds because a contaminant from the SEP-PAK resin cartridge was found to coelute with ATR and prevented ATR quantification. In method I, all pesticides (except ATR) were resolvable using a three-stage temperature programming ramp where the initial column temperature was held for 2 min at 60°C, then raised to 225°C at 15°C min^{-1}, and finally raised to 250°C at 4°C min^{-1} and held for 20 min. In method II, a three-stage temperature ramp was also used to resolve ATR from the contaminant. The initial column temperature was the same; but the temperature was raised to 180°C at 12°C min^{-1} and held for 3 min, followed by an increase to 195°C at 3°C min^{-1} and held for 21 min. This ramping allowed the contaminant, ATR, and TBA peaks to be separated with a difference in relative retention times by approximately 8 to 9 s between peaks. In method II, the oven temperature reduction successfully separated the cartridge contaminant from ATR, but also resulted in the coelution of AMET and METAL. We were, therefore, unable to resolve all 12 pesticides just using method II.

A good linear response ($r^2 > 0.99$) in the range of 0 to 300 μg L^{-1} was achieved for all GC runs. Quantification was performed by calculating a peak area ratio of pesticide of interest divided by the TBA (internal standard) peak area and comparing to standard peak area ratios. The minimum detection limits (MDL), as shown in Table 2, were calculated using pesticide standards to verify lower detection limits.

TABLE 2
Chromatographic Characteristics of Select Agricultural Pesticides and Metabolites.

Pesticide	Retention time (min)†		MDL (μg L^{-1})‡	
	$\bar{x}$	s	$\bar{x}$	s
Method I				
DEA	13.31	0.09	0.15	0.03
DIA	13.39	0.09	0.15	0.04
PMT	13.52	0.09	0.20	0.15
TBA§	13.75	0.03	--	--
ALA	15.03	0.10	0.35	0.18
PRYN	15.25	0.11	0.15	0.06
AMET	15.39	0.11	0.15	0.07
METAL	15.47	0.11	0.30	0.05
METRIB	15.53	0.11	0.20	0.08
METOL	15.73	0.11	0.30	0.19
CYAN	16.87	0.13	0.10	0.01
Method II				
ATR	18.50	0.16	0.15	0.02
TBA§	18.65	0.09	--	--

† Results determined from 12 replicate 1.8-μL injections of 300-μg L^{-1} spike sample.

‡ MDL were determined from 6 replicate 1.8-μL injections of a spiked sample with a peak signal:noise ratio of 3:1 and a 50:1 concentration factor.

§ Used as internal standard.

A 1.8-μL injection volume was used for all injections, which corresponds to 9 to 1500 pg of analyte on column.

Field Sample Preparation and Pesticide Recovery Determination

Stream grab samples were periodically collected during 1994 and 1995 from several locations around the Herrings Marsh Run watershed in Duplin Co., North Carolina. The streams of this watershed are typical Coastal Plain streams having dissolved organic carbon (DOC) concentrations between 10 and 20 mg L^{-1} and pH levels between 5 and 6. Samples were immediately packed in ice and transported back to the laboratory. All field samples were maintained frozen until analyses. Samples were thawed at room temperature (22-25°C) and mixed by hand shaking. Method extraction efficiency was determined by randomly selecting one stream sample and subsampling two 50-g portions into separate beakers. One portion served as the spike sample by adding 1.0 mL of 100-μg L^{-1} pesticide spiking solution, shaking gently for a few seconds, then vacuum filtering through a Whatman (Clifton, NJ) GF/F glass microfiber filter (0.7-μm avg. pore diameter) to remove suspended solids. The other portion served as the unspiked control and was filtered in a similar manner. Both unspiked and spiked samples were extracted using the SPE method as described below. Correction for background pesticides was assessed from the unspike control. Recovery percentages for each pesticide were determined by examining the ratio of pesticide concentration in the spike control to pesticide concentration in the spiking solution. This procedure was repeated 12 times with actual field stream samples.

SPE Cartridge Activation and Pesticide Extraction

All pesticides were extracted using a Waters tC_{18} plus SEP-PAK (Waters Co., Milford, MA) SPE cartridge (390-mg sorbent bed) on a Visiprep Solid Phase Extraction Vacuum Manifold (Model 5-7030M; Supelco, Inc., Bellefonte, PA). Reports of coeluting contaminants from the SPE cartridge (Junk et al., 1988; Watts et al., 1994) prompted an extensive clean-up procedure where each cartridge was prerinsed three times with 1-mL portions of EtOAc under a partial pressure of 3×10^{-2} MPa. After prerinsing, the SPE cartridge was activated under partial vacuum (3×10^{-2} MPa) by passing one 10-mL portion of MeOH, followed by a 10-mL rinse of H_2O. Cartridges were not allowed to dry during the activation cycle.

A 50-g filtered aliquot of each stream sample was then passed through the activated SPE cartridge under vacuum at a flow rate that did not exceed 5 mL min^{-1}. After the aliquot passed through the SPE cartridge, the cartridge was rinsed with 10 mL of H_2O and dried under full vacuum (6.6×10^{-2} MPa) for 30 min.

Sorbed pesticides were then eluted from each SPE cartridge by passing 3 x 1-mL portions of EtOAc into a 15-mL conical glass centrifuge tube. The bulk of the EtOAc was allowed to pass through under gravity, then any remaining portions were removed under full vacuum for approximately 5 min. Each centrifuge tube was spun at a sufficient speed to separate any residual H_2O from EtOAc. After the residual H_2O was removed using a pasteur pipet, each tube was placed into a heating block. The EtOAc in each tube was allowed to evaporate to < 1 mL under N_2 (g) and low heat (40°C). Each sample was adjusted to 1.0 mL using EtOAc then spiked with 0.02

mL of 5100-μg TBA L^{-1}, which corresponded to an internal standard concentration of 100-μg TBA L^{-1}.

A MeOH induced matrix effect resulted in coelution of AMET and METAL when the GC spiking solution was prepared in 100% MeOH. A modified method whereby 1 mL of the GC spiking solution was evaporated to near dryness and was reconstituted to 1 mL with 100% EtOAc eliminated the coeluting problem. By reducing the MeOH to be < 25% (v/v), we were able to resolve the AMET and METAL peaks.

Quality Control Protocol

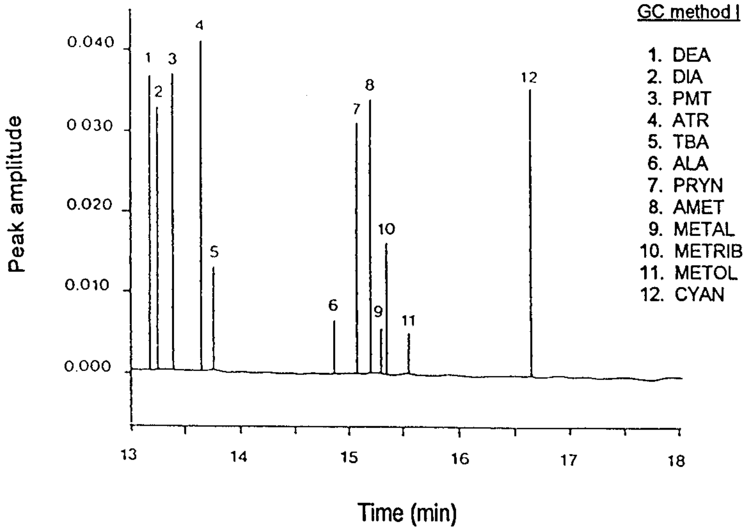
A rigorous quality control procedure was implemented throughout the SPE extraction and GC quantification steps. All glassware was thoroughly washed and rinsed with Milli-Q H_2O, and a final rinse of HPLC-grade MeOH was done to insure the removal of any pesticide residues. All solvents were examined for trace amounts of pesticides prior to use. All GC standards were remade monthly. The SPE cartridges were tested during each batch extraction for the presence of pesticides or coeluting contaminants. The reproducibility of our method was tested by performing multiple (n = 3) extractions and analyses during different months. Our minimum detection limits (MDL) were tested monthly (n = 6) by directly injecting trace amounts (0 to 10 μg L^{-1}) of each pesticide into the GC. The MDLs were determined from 1.8-μL injections of spiked standards, arbitrary selection of peak signal:noise ratio of 3:1, and use of a 50:1 concentration factor to estimate the limit. Periodically, pesticide concentrations in select standards and samples were confirmed by Dr.

Richard Pfeiffer (USDA-ARS-NSTL) using a gas chromatography/mass spectrometry (GC/MS) in the selective ion mode as described in Pfeiffer and Steinheimer (1992).

RESULTS AND DISCUSSION

Our ability to resolve pesticide and metabolite peaks on the GC was influenced by the solution matrix. When the GC spiking solution prepared in 100% MeOH was directly injected into the GC, AMET and METAL peaks both coeluted. The MeOH apparently caused AMET and METAL to have a similar chemical behavior with the functional groups of the Rtx-35 capillary column and to consequently coelute. Our method was modified so that during each batch extraction, a 1-mL subsample of the GC spiking solution was evaporated almost to complete dryness and brought back to volume with EtOAc. This reduced the volume of MeOH in the matrix to < 25% (v v^{-1}). This modification prevented coelution of AMET and METAL peaks on the GC column.

Figure 1 shows a typical chromatogram of a 300-$\mu g\ L^{-1}$ spiked sample containing the 11 compounds and TBA (internal standard) resolved using both GC method I (top) and method II (bottom). In the first chromatogram, all 12 compounds were resolvable using method I. There was no coeluting contaminant in this spiked sample since it was not passed through the SPE cartridge. Our GC method II revealed the separation of the ATR, cartridge contaminant, and TBA peaks. Leaching the SPE cartridges with 4 to 6 additional 1-mL portions of EtOAc did not remove the coeluting contaminant. It was decided to modify the GC method to resolve ATR from the contaminant whereby the temperature ramp was slowed down to adequately



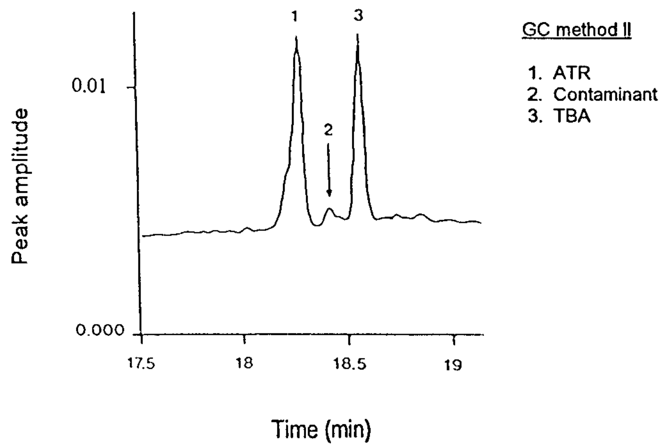


FIGURE 1
GC Chromatogram Showing Pesticides and Metabolites Separated Using GC Method I (top) and GC Method II (bottom).

separate the contaminant peak from the ATR peak. When we modified the temperature ramps in method I to adequately separate the cartridge contaminant from the ATR peak, the later eluting pesticides coeluted. Therefore, ATR was quantified using GC method II while the remaining select pesticides were quantified using GC method I. The appearance of the coeluting contaminant illustrates a serious drawback of using the SPE cartridges for pesticide extraction and quantification.

The elution order of the pesticides and metabolites revealed the relative degree of compound polarity with respect to the mid-polar stationary phase of the GC capillary column. The triazine metabolites DEA and DIA were the first to elute, with cyanazine eluting last (Table 2). The DEA and DIA are slightly more polar than ATR and will elute more rapidly from the mid-polar GC column. The more polar triazines (AMET, TBA, CYAN, etc) and chloroacetamides (ALA and METOL) migrate through the column at different times because of the different degree of interaction between the column stationary phase and the compound itself. The Rtx-35 capillary column was quite sufficient to resolve all compounds.

The mean MDLs for all pesticides ranged from 0.10 to 0.30 $\mu g\ L^{-1}$ (Table 2). They were calculated by making injections of very dilute pesticide-spiked solutions, obtaining the predicted pesticide concentrations, and dividing these concentrations by 50. This concentration factor was used because our procedure involved extracting 50 mL of water and reducing to 1-mL final weight. The low MDLs of several pesticides were due to the occurrence of several N atoms in the molecule, which consequently increases the compound's response to the NPD. Cyanazine, with the lowest MDL (0.1 $\mu g\ L^{-1}$), has 6 N atoms in its structure, which resulted in the greatest response to the NPD detector (Table 2). In contrast, ALA, METAL, and METOL, with only 1 N atom in the molecule, had the highest MDLs. Our MDLs in this study imply that we can expect to quantify trace amounts (between 0.10 and 0.35 $\mu g\ L^{-1}$) of these pesticides in our Coastal Plain stream samples using this method.

Our method recovery efficiency of extracting, concentrating, and quantifying a diverse mixture of pesticides and metabolites from stream water samples was good. The mean recoveries ranged from 19% to 98%, with the bulk of the pesticide recoveries being > 85% (Table 3). The SPE and GC procedure was not affected by the relatively high concentration (10-20 $mg{\cdot}L^{-1}$) of DOC typically present in the stream samples. The high recovery of most pesticides implies that the triazines and chloroacetamides family of pesticides may be adequately extracted from stream samples using the SEP-PAK tC_{18} plus cartridge. On the other hand, the atrazine metabolites (DEA and DIA) mean recoveries are lower than the other compounds. Low recoveries of DEA using this same type of SPE cartridge were also reported by Nash (1990) who recovered only 26% DEA from spiked samples. The low recoveries of the metabolites could be related to their hydrophilic nature, which contributes to poor retention in the MeOH-enriched microenvironment around each C_{18} functional group in the cartridge. With respect to the pesticides other than DEA and DIA, our method recovery efficiencies are of similar magnitude to Thurman et al. (1990) and Nash (1990) who extracted and recovered a mixture of pesticides using a similar SPE-type cartridge.

Repetitive extractions to evaluate the reproducibility of predicting pesticide concentrations in samples were accomplished by selecting an unspiked stream sample

TABLE 3
Recoveries of Selected Agricultural Pesticides and Metabolites from Spiked Stream Samples[†]

Pesticide	$\bar{x}$	S
Method I	%	
DEA	60	10
DIA	19	7
PMT	88	14
ALA	94	11
PRYN	85	7
AMET	87	7
METAL	99	9
METRIB	92	14
METOL	85	13
CYAN	98	8
Method II		
ATR	98	16

[†] Recoveries determined from 12 separate extractions.

with detectable amounts of ALA, METRIB, and CYAN (Table 4). This stream sample was extracted on three separate occasions. Mean ALA, METRIB, and CYAN predicted concentrations in this sample had excellent reproducibility with coefficients of variations that ranged from 4% to 13%. The pesticides in this water sample were confirmed by GC/MS, and the predicted concentrations for these pesticides were similar (variation < 10%).

TABLE 4
Repeated Extractions to Evaluate Reproducibility of Predicted Pesticide Concentrations in a Stream Sample.[†]

	GC					
Pesticide	Rep A	Rep B	Rep C	$\bar{x}$	S	%CV
	---------	$\mu g\ L^{-1}$	---------			
ALA	9.75	9.31	7.61	8.90	1.13	13
METRIB	1.30	1.40	1.40	1.36	0.06	4
CYAN	2.60	2.52	2.92	2.70	0.21	8

[†] Stream samples spiked only with TBA.

Our SPE and capillary GC pesticide extraction procedure was found to be rapid and to have a high degree of sensitivity. Trace levels of pesticides in an actual stream sample were extracted, and the degree of pesticide concentration prediction was quite reproducible. This method may be used to extract and quantify commonly used pesticides and some metabolites in stream samples with a high degree of accuracy.

ACKNOWLEDGEMENTS

Sincere thanks are expressed to Dr. Richard Pfeiffer at the USDA-ARS-NSTL for the GC/MS sample extraction and analyses.

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Received: March 20, 1996